MONOCYTIC LYSOZYME LEVELS IN MALIGNANT DISEASE

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ABSTRACT

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By

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Intracellular monocytic lysozyme levels were measured from three groups of individuals: patients with untreated malignant disease, patients with benign disease, and normal individuals as a control group. The patients with untreated malignant disease had an elevation of their monocytic lysozyme content.

The mononuclear leukocyte layer of cells was separated by the Ficoll-Hypaque procedure. A cell count was done on the product and a Wright's stain was used to determine the percentages of monocytes and lymphocytes.

The cell preparations were lysed by 5 cycles of freezing and thawing. The preparations were checked by phase contrast microscopy to determine that all cells were lysed.

The lysozyme levels were measured by the lysoplate method at 25°C. The turbidimetric method was investigated and found to be unacceptable for the concentration at which the cells had been suspended (10^7 monocytes per ml).

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By

Nancy R. Jaehn

A THESIS

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In memory of my parents,

Harold and Clara Jaehn

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INTRODUCTION

Lysozyme was discovered in 1922 by Alexander Fleming when he observed that it caused lysis of some bacterial cell walls. He found lysozyme to be present in many tissues and secretions of the human body.

Recent studies have investigated the possibility that certain polysaccharides of mammalian cells may be susceptible to enzymatic cleavage or substrate specific binding by lysozyme. E. F. Osserman has postulated that lysozyme may participate in the surveillance of membrane abnormalities, particularly those associated with neoplastic formation. Lysozyme may be a key mediator of the anti-tumor functions of macrophages.

The blood-borne precursors of tissue macrophages are monocytes. This research measured the lysozyme concentrations in human monocytes in patients with untreated malignant disease, patients with benign diseases, and normal individuals as a control group. The primary objective was to determine if there is a significant relationship between intracellular monocytic lysozyme levels and malignant disease.

The Ficoll-Hypaque technique was used to separate the mononuclear leukocyte layer from the other blood constituents. A cell count was done to determine the number of cells

present, and a Wright's stain differential was done to determine the percentages of lymphocytes and monocytes present. The cell preparations were lysed by 5 cycles of rapid freezing and thawing. Both the turbidimetric and the lysoplate method were used to measure the lysozyme activity.

LITERATURE REVIEW

This literature review will be divided into three parts: (1) lysozyme - discovery, chemical activity, structure, multiple forms, and function; (2) monocytes-macrophages in neoplasia - role in non-specific cell mediated immunity, effect of Bacillus Calmette-Guerin (BCG) and *Corynebacterium parvum*, and lysozyme concentrations in activated macrophages-monocytes; (3) lysozyme methodology - several observations regarding the turbidimetric and lysoplate methods.

Lysozyme

Discovery

Lysozyme was discovered by Alexander Fleming in 1922 when he observed that it caused lysis of some bacterial cell walls. He found lysozymes to be present in many tissues and secretions of the human body, such as tears, saliva, leukocytes, and skin. Fleming postulated lysozyme to be the natural defense agent of the human body. It was the first bactericidal substance he had studied that fulfilled a major requirement: that it be selectively more lethal to bacteria than to host cells (Fleming, 1922).

Chemical Activity

Lysozyme hydrolyzes the β 1,4 glycosidic linkages between N-acetylglucosamine and N-acetylmuramic acid in bacterial cell walls. It may be of animal, plant, or microbial origin (Osserman, 1974).

The oxygen atoms of the aspartic 52 and glutamic 35 residues of lysozyme specifically accomplish the hydrolytic cleavage (Phillips, 1967).

Lysozyme is a small cationic protein molecule with a molecular weight of 14,000. Its optimum pH range is 6.0-6.5. It is heat stable at temperatures up to 52°C. Lysozyme specimens can be stored at -20°C for several weeks (Daniels, 1974). The lysozyme molecule is stable to repeated freezing and thawing (Gordon et al., 1974; Perillie, 1968; Hansen and Andersen, 1973).

Lysozyme Structure

In 1965 Phillips and his co-workers produced an electron density map of lysozyme, and at the same time Jolles, P. (1964) in Paris and Canfield (1965) at the National Institutes of Health with their colleagues determined the amino acid sequence of the enzyme. This enabled them to identify the conformational features of the molecule by looking at the map in relation to the amino acid sequences.

The lysozyme molecule consists of two immunologically active components. One immunologically active fragment consists of two peptides derived from the amino terminus

(residues 122-129) linked together by a disulfide bond. The other immunologically active fragment is derived from the region between residues 57-107 with two disulfide bonds. This also encompasses the area referred to as the "loop" of residues 60-83 (Arnon, 1969).

Multiple Forms

Only a single form of lysozyme was found in hen egg-white and goose egg-white as well as in normal human secretions and tissues. In the latter, lysozyme was purified from tears, leukocytes, plasma, milk, and placenta. The enzymes from the different sources were found to have the same chromatographic and electrophoretic behaviors, as well as the same amino acid sequences (Jolles, P., 1969).

Multiple enzymes have been found in duck egg-white (Jolles, J., 1965), and in the tissues and secretions of patients with leukemia (Mouton, 1969).

Immunologic methods can provide estimates of the approximate degree of sequence resemblance and conformational similarity among the related lysozymes (Prager and Wilson, 1971).

Function

The function of lysozyme in higher vertebrates, other than its probable antibacterial role, is still not clear.

The possibility has been suggested that lysozyme may have other important physiological functions, some of which may be related to specific enzymatic activities on

nonbacterial substrates, and others with its marked cationic property (Ambrose, 1966).

It has also been postulated that lysozyme may be a key mediator of the anti-tumor functions of macrophages (Osserman, 1974).

The presence of macrophages in tumors of man has been documented (Hamlin, 1968), and there is considerable evidence that these cells serve a major role in host defenses against neoplasia (Halpern, 1953; Old et al., 1961; Holterman et al., 1972).

Since monocytes are the blood-borne precursors of macrophages (Osserman, 1966; Lessin and Bessis, 1972; Gordon et al., 1974), this research measured the level of lysozyme in peripheral blood monocytes.

Clinical and experimental studies indicate that from 70 to 80% of the serum and tissue lysozyme is within or released from neutrophils and monocytes (Finch et al., 1974).

Farhangi and Osserman (1974) have found that monocytes release their enzyme after synthesis, while granulocytes retain their lysozyme probably within lysosomes and release it only in the course of lysosomal disruption. This would imply that there may be two different cell populations (cells of the monocytic-macrophage series and those of the granulocytic series) which utilize the same enzyme, lysozyme, in two distinctly different ways. That is, there may be specific biological functions of lysozyme as mediated by these different cell types.

The kidney plays a significant role in the degradation of lysozyme (Finch et al., 1974).

Monocytes-Macrophages in Neoplasia

Role in Non-Specific Cell-Mediated Immunity

The role of large mononuclear leukocytes in host defense against neoplastic cells is receiving increased attention. It has been suggested that such leukocytes are effectors in a primitive surveillance mechanism against aberrant cells (Holterman, 1973; Osserman, 1974).

A phenomenon possibly related to a local amplification of this primitive surveillance mechanism in man is regression of cutaneous neoplasms after recruitment of mononuclear inflammatory infiltrates at the tumor sites by induction of delayed type hypersensitivity reactions to chemical haptens (Klein, 1969), or by lymphokines (Holterman, 1974; Papermaster, 1974).

Increased resistance to carcinogens and transplanted tumors after administration of various agents with a stimulatory effect on the reticuloendothelial systerm (Yashphe, 1971) may be a reflection of a systemic activation of the same mechanism. This implies that large mononuclear cells are selectively cytotoxic for neoplastic cells.

Zbar (1974) describes the current understanding of the cellular immune response which kills tumor cells when mediated by a non-specific stimulator such as Bacillus Calmette-Guerin (BCG), a bovine strain of *Mycobacterium*.

Bacillus Calmette-Guerin is a potent non-specific stimulator of the reticuloendothelial system which has caused tumor regression. Following the infection of the host, the host develops lymphocytes capable of recognizing the distinctive antigens of the tubercle bacillus. The lymphocytes react with the bacillus and produce a variety of potent molecules. Some of these may be directly cytotoxic. If tumor cells are anatomically close to the bacteria, the tumor cells are killed by the activated macrophages and lymphotoxins. As a consequence of this reaction, immunity to specific tumor antigens may develop and, in turn, produce additional tumor cell death.

Corynebacterium parvum has recently been found to have tumor resistance-inducing properties in experimental animals (Currie, 1970) and in preliminary studies with humans (Dimitrov, 1974).

Activated macrophages are characterized by increased phagocytosis and increased content of lysosomal enzymes (Eisen, 1974).

Since the large mononuclear cells of the peritoneum and other tissues, at least in part, are derived from the monocytes of the blood, one might expect that monocytes would also exhibit cytotoxic properties. Human blood monocytes were reported as cytotoxic to cells of a human osteosarcoma and a reticulum cell sarcoma *in vitro*. Activation of the monocytes by supernatant fluids of mitogen stimulated human lymphocytes markedly increased their cytotoxicity towards neoplastic cells (Holterman, 1974).

There does not seem to be any limit on the type of tumor susceptible to increased non-specific stimulation of host resistance. It would appear that tumors must be antigenic to be susceptible to such effects (Yashphe, 1971). Yashphe designates a non-specific stimulator as a material whose administration confers on the recipient the ability to respond, or respond more efficiently, to challenge with a non-cross reacting antigen, bacterium, or tumor as measured either by a heightened specific immune response or by increased resistance. According to Yashphe the best results of therapy against malignant disease may be based on the non-specific activation of the organism's responsiveness to the tumor cells, rather than focusing attention on specific immunotherapy by means of tumor associated antigens.

Lysozyme Concentrations in Activated Macrophages-Monocytes

Carson and Dannenberg (1965) reported lysozyme levels 1.4 times normal in peritoneal macrophages collected from tuberculous rabbits.

Lysozyme levels in extracts of alveolar macrophages from rabbits vaccinated and challenged with tubercle bacilli of the BCG strain were 5 to 6 times normal (Oshima et al., 1961). The authors postulate that the high content of lysozyme in alveolar macrophages from normal rabbits may be related to the capacity of the cells to destroy some of the microorganisms which commonly invade the lower respiratory tract.

The fact that lysozyme is elaborated by and released from macrophages is well documented (Myrvik et al., 1961; Cohn and Wiener, 1963). An increased production and/or release of lysozyme by macrophages following BCG vaccination has also been demonstrated (Cohn and Wiener, 1963; Myrvik et al., 1962; Carson and Dannenberg, 1965).

Osserman (1974) has found that lysozyme alters the structure and *in vitro* behavior of certain transformed mammalian cells and postulates that lysozyme may be one if not the major mediator of the anti-tumor functions of macrophages.

Review of Lysozyme Methodology

Perillie and Finch (1974) and Hansen and Andersen (1973) comment on the lack of agreement between the two methods of analysis of lysozyme, and note the use of the two commonly employed standards, egg-white and human lysozyme.

The method generally employed is a turbidimetric procedure in which clearing of a suspension of *Micrococcus lysodeikticus* occurs in proportion to the concentration of lysozyme present in the test specimen (Osserman, 1966).

There is also a radial diffusion (lysoplate) method (Osserman, 1966), in which heat-killed *Micrococcus lysodeikticus* organisms are suspended uniformly in molten agar and poured into petri dishes. Wells are cut in the agar for the test samples. Zones of clearing develop in the gel as the result of bacterial lysis. The diameter of the

cleared zone is proportional to the concentration of lysozyme in the test specimen.

METHODS AND MATERIALS

Specimen Selection

Twelve patients with malignant disease were selected prior to their chemotherapy program. Eleven patients with a variety of benign conditions (defined as those conditions which are not malignant) were selected as a comparison group. Fourteen individuals free of known disease were included as a control group.

The groups were age and sex matched as closely as feasible. The patients and normal individuals did not have symptoms of renal disease. To eliminate this possibility serum BUN or creatinine levels were measured on each specimen.

Preparation of the Monocytes

Fifty milliliters of EDTA anticoagulated blood and 5 ml of blood allowed to clot were drawn from each patient or control and were processed immediately. The collecting apparatus consisted of a 19-gauge butterfly needle and a 50 ml plastic syringe.

A total white blood cell count and differential were done on each whole blood specimen.

The mononuclear leukocyte layer of cells was obtained using the gradient centrifugation technique employing

Ficoll-Hypaque* according to the method of Boyum (1968).

The 50 ml of EDTA anticoagulated blood were diluted 1:1 with 0.9% NaCl, pH 7.1. Seven milliliters of Ficoll-Hypaque solution were layered under 20 ml of the diluted blood. This was centrifuged immediately for 20 minutes at 650 g. The monocytes and lymphocytes appeared as a white ring on top of the Ficoll-Hypaque layer. The monocytes and lymphocytes were removed with a capillary pipette and placed in a 50 ml plastic centrifuge tube. The cells were washed three times in 0.9% NaCl. Centrifugation was at 300 g for 10 minutes for each wash.

All plastic containers were used since monocytes adhere to glass. It was necessary to process rapidly and mix well since the cells tended to clump.

Immediately following the final wash a cell count was done using the Coulter Fn automatic cell counter.** A minimum of three smears of the final product were made. Approximately equal amounts of the cell preparation and the patient's serum were mixed and spread on a glass slide. The serum is necessary to insure good staining of the cells. The slides were stained on an Ames Hematek automatic slide stainer*** which utilized a Wright's-Giemsa stain.

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*Ficoll, F-4375, Sigma Products, St. Louis, MO; Hypaque (50%), NDC 24-H65-4, Winthrop Labs, New York, NY.

**Coulter Electronics, Hialeah, FL.

***Ames Company, Elkhart, IN.

A minimum of three differentials were done for each cell preparation and the respective percentages of monocytes, lymphocytes, and granulocytes (if any) were determined.

The cell preparation was then adjusted to the proper volume so that all specimens had a concentration of 10^7 monocytes/ml of cell preparation.

Several attempts were made to separate the monocytes from the lymphocytes. By utilizing the characteristic of adherence possessed by the monocytes, two techniques were investigated.

The method of Brodersen and Burns (1973) involved incubating the mononuclear cell preparations in small glass beakers at 37°C on a shaker in a constant 5% CO_2 environment. After two hours the supernatant and nonadherent cells were decanted. The adherent monocytes were obtained by gentle scraping of the walls of the beakers.

The second technique involved passing the cell preparation through a column of glass beads according to the method of Rabinowitz (1965). The monocytes adhere to the glass beads and the lymphocytes pass through. Adherent cells are then released from the glass beads with 0.02% EDTA in Ca^{++} and Mg^{++} free buffered saline.

In both of these instances the yield of monocytes obtained was inadequate. Generally these methods are successful when a full unit (500 ml) of blood is drawn. Since it was not possible to obtain this quantity of blood from patients with malignant disease, and since it is well

documented that lymphocytes do not contain lysozyme (Flanagan and Lionetti, 1955; Gordon et al., 1974), it was decided not to attempt to remove the lymphocytes.

Disruption of the Cell Membrane

The method of choice was five cycles of rapid freezing at -20°C and thawing at room temperature (25°C). Ohta and Osserman (1972), Gordon et al. (1974), and Perillie (1968) all successfully utilized this method.

Phase contrast microscopy was used to determine that all cells had been disrupted.

Cell membranes can also be disrupted by means of sonication. A Branson model 140 sonicator* was utilized for this purpose. However, due to the high temperature (80-90°C) which develops within the specimen during sonication, the lysozyme molecule was apparently destroyed. When lysozyme determinations were made on standard solutions following sonication, activity was lacking in the low concentrations and greatly diminished in the higher concentrations. A cooling jacket for the specimen container may have alleviated the problem, but this was not available at the time of the experimentation.

Lysozyme Analysis by the Lysoplate Method

The radial diffusion or lysoplate method (Osserman, 1966) utilized heat-killed *Micrococcus lysodeikticus*

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*Heat Systems Ultrasonics, Inc., Plainview, Long Island, NY.

organisms suspended uniformly in 1% molten agar. This mixture was poured into petri dishes. Wells were cut in the agar, and the test sample was added to the well. Recently the lysoplates became commercially available* and these plates were utilized in this research.

A Sherwood pipette** with disposable plastic tips was used to deliver 10 µl of the lysed monocyte cell preparation to the test wells. This must be done with care so all the specimen enters the well, and bubbles and splatters are avoided. All test specimens were run in triplicate.

A reference enzyme solution was prepared by dissolving human lysozyme standard (urine)*** at a concentration of 1000 µg/ml in sodium phosphate buffer, pH 7.0. The reference enzyme solution was further diluted with sodium phosphate buffer to produce standards of the following concentrations (µg/ml): 150, 100, 50, 25, 15, 10, and 5.

The plates were incubated at room temperature (25°C) for 18 hours. Zones of clearing develop in the gel as a result of bacterial lysis. The diameter of the cleared zones was measured to the nearest 0.5 mm. Indirect light was used to enhance the contrast and facilitate reading of the plates.

The diameter of the cleared zones is related logarithmically to the enzyme concentration. Using semilogarithmic

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*RADL, 3483, Worthington Biochemical Corp., NJ.

**Sherwood Medical Industries, St. Louis, MO.

***LYHU92910, Worthington Biochemical Corp., NJ.

paper, the zone diameter (in mm) of each standard concentration was plotted on the linear axis. The standard enzyme activities (in µg/ml) were plotted on the log axis.

The activities of the cell preparations were read from the standard curve prepared as above.

This value was corrected for granulocyte contamination (all test specimens had 10% or less granulocyte contamination). Several authors indicate that monocytes and granulocytes have approximately equal amounts of intracellular lysozyme (Senn et al., 1970; Hansen and Andersen, 1973; Ohta and Osserman, 1972), although their exact numerical values vary due to different methodologies and the use of different standards.

For example, a specimen with 2% granulocytes and 34% monocytes (the remainder lymphocytes not containing lysozyme) with a lysoplate reading of 65 µg lysozyme/10^7 cells per ml would be corrected as $\frac{34}{36}$ x 65 = 61 µg lysozyme/10^7 cells per ml.

Lysozyme Analysis by the Turbidimetric Method

The turbidimetric method utilizes a suspension of *Micrococcus lysodeikticus* cells in sodium phosphate buffer, pH 6.2.* A clearing of the suspension occurs which is proportional to the concentration of lysozyme in the test specimen.

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*RLK #7978, Worthington Biochemical Corp., NJ.

Three milliliters of the *Micrococcus lysodeikticus* substrate suspension were pipetted into a cuvette of the Gilford 240 Spectrophotometer.* Distilled water was used for the blank. The reaction was measured at 25°C, and at a wavelength setting of 550 nm. Three-tenths milliliter of serum or appropriate dilution of test specimen was added to the substrate. Absorbance readings were taken at 30 seconds and at three minutes. The change in absorbance (ΔA) over the two and one-half minute time interval was determined by the difference between the two absorbance readings.

Worthington Biochemical Corporation supplies an egg-white standard for use with this procedure. The standard has a lysozyme concentration of 40 µg/ml. Appropriate dilutions were made with distilled water to provide the following recommended standards (in µg/ml): 20, 16, 12, 8, 4, and 2. The concentration of the standards was plotted on the horizontal axis, and the ΔA value was plotted on the vertical axis. The concentrations of the test specimens were then read from the curve.

It is necessary to repeat on appropriate dilutions any specimens with lysozyme concentrations greater than 16 µg/ml, since the reaction drops off rapidly at this point due to depletion of the substrate. Since the reaction is not linear over the two and one-half minute interval

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*Gilford Instrument Laboratories, Inc., Oberlin, OH.

(personal observation) specimens run on dilution are susceptible to increased error.

In this project all sera were assayed by this method. Since the serum lysozyme values are generally under 15 µg/ml, this method provided the best sensitivity.

Egg-White Standard vs. Human Standard

The lysozyme standard is assayed by determining the rate of lysis of *Micrococcus lysodeikticus* cell suspensions. One unit is defined as that which causes a decrease in turbidity of 0.001 per minute at 450 nm at pH 7.0 and 25°C. A wide range of activities are reported for pure lysozyme preparations under these conditions (Worthington Biochemical Corp., 1976).

The Worthington egg-white standard assay is established in relationship to a high purity lysozyme standard defined at 12,000 units/mg protein. The egg-white standard used in this research had an activity of 9600 units/mg as assayed by Worthington.

The Worthington human standard is isolated from the urine of patients with monocytic leukemia. The activity is approximately three times greater than the egg-white standard. The human standard used in this research had an activity of 33,500 units/mg as assayed by Worthington.

Due to the high activity of the human standard, the substrate was depleted much too rapidly for use by the turbidimetric method. The 5 µg/ml human standard had a

ΔA of 0.074. The 10 µg/ml had a ΔA greater than 0.100, which indicates the substrate has been depleted.

However, the human standard is an ideal choice for the lysoplate method since the lysoplates have their best sensitivity from 25 to 500 µg/ml. Also, the human standard eliminated any differences in migration of the lysozyme in the lysoplates which may have been a factor in the use of an egg-white standard since their chemical conformations do vary.

RESULTS AND DISCUSSION

Monocytic Lysozyme Levels as Measured by the Lysoplate Technique with Human Standard

The results of the monocytic lysozyme levels of the patients with malignant or benign disease and the normal control individuals are illustrated in Figure 1.

The patients with malignant disease had a significantly higher monocytic lysozyme content (mean 70 $\mu g/10^7$ cells) than the normal individuals (mean 41 $\mu g/10^7$ cells) or patients with benign diseases (mean 39 $\mu g/10^7$ cells).

Ohta and Osserman (1972) obtained a mean lysozyme content of normal monocytes of 3.0 $\mu g/10^6$ cells, which is very similar to the present findings. They also utilized the lysoplate method and the human standard.

Figure 2 is the lysoplate standard curve with human lysozyme standard. The human standard is the one of choice since it eliminates any question of variability of migration due to the conformation of the molecule which occurs when the egg-white standard is used.

It can also be noted from Figure 2 that the lysoplate method is useful for substances with relatively high concentrations of lysozyme in the range of 25-150 µg lysozyme per ml.

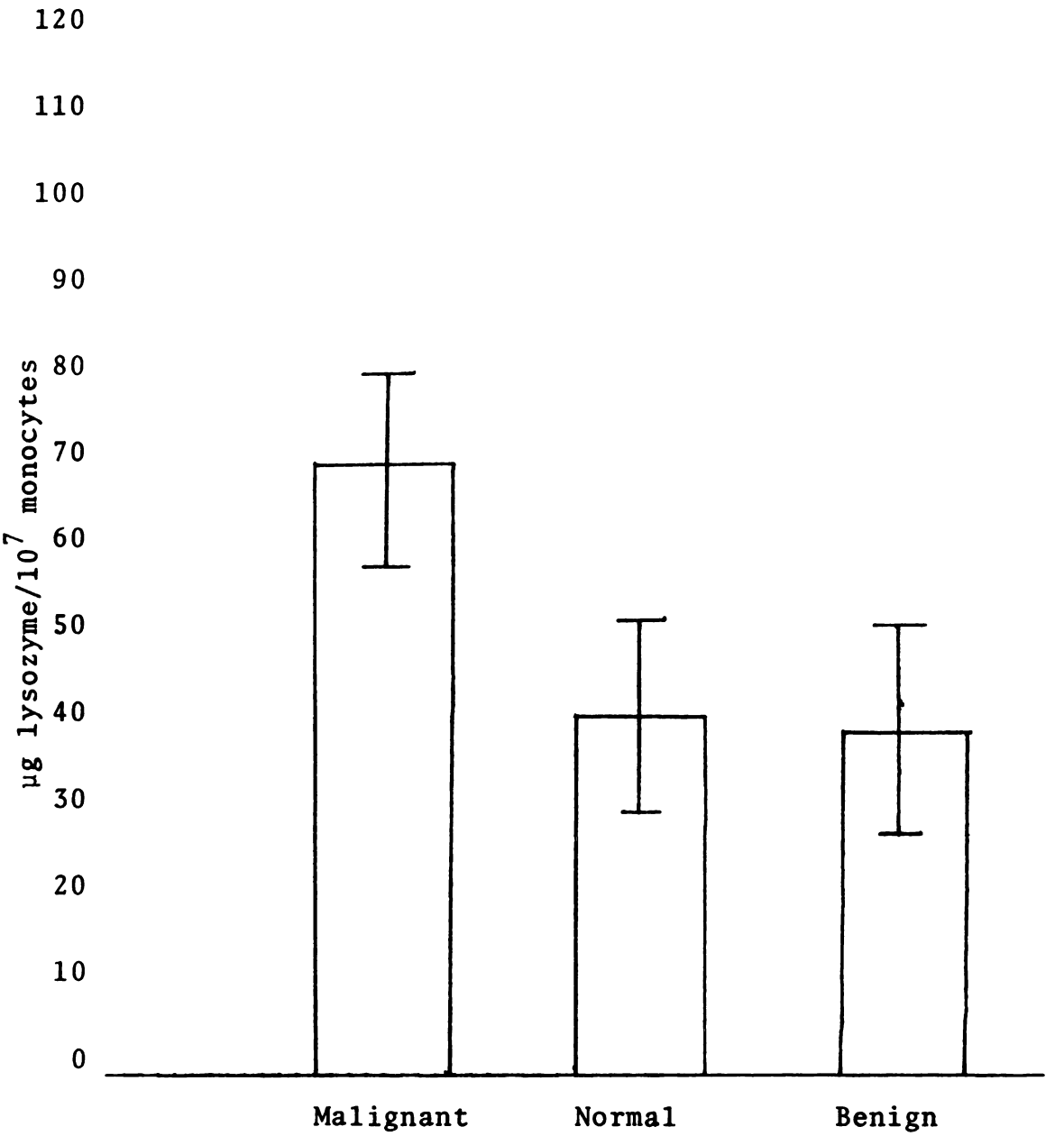

Figure 7. Comparison of mean intracellular monocytic lysozyme in patients with untreated malignant disease, in normal individuals and in patients with benign diseases. Brackets indicate 95% confidence intervals.

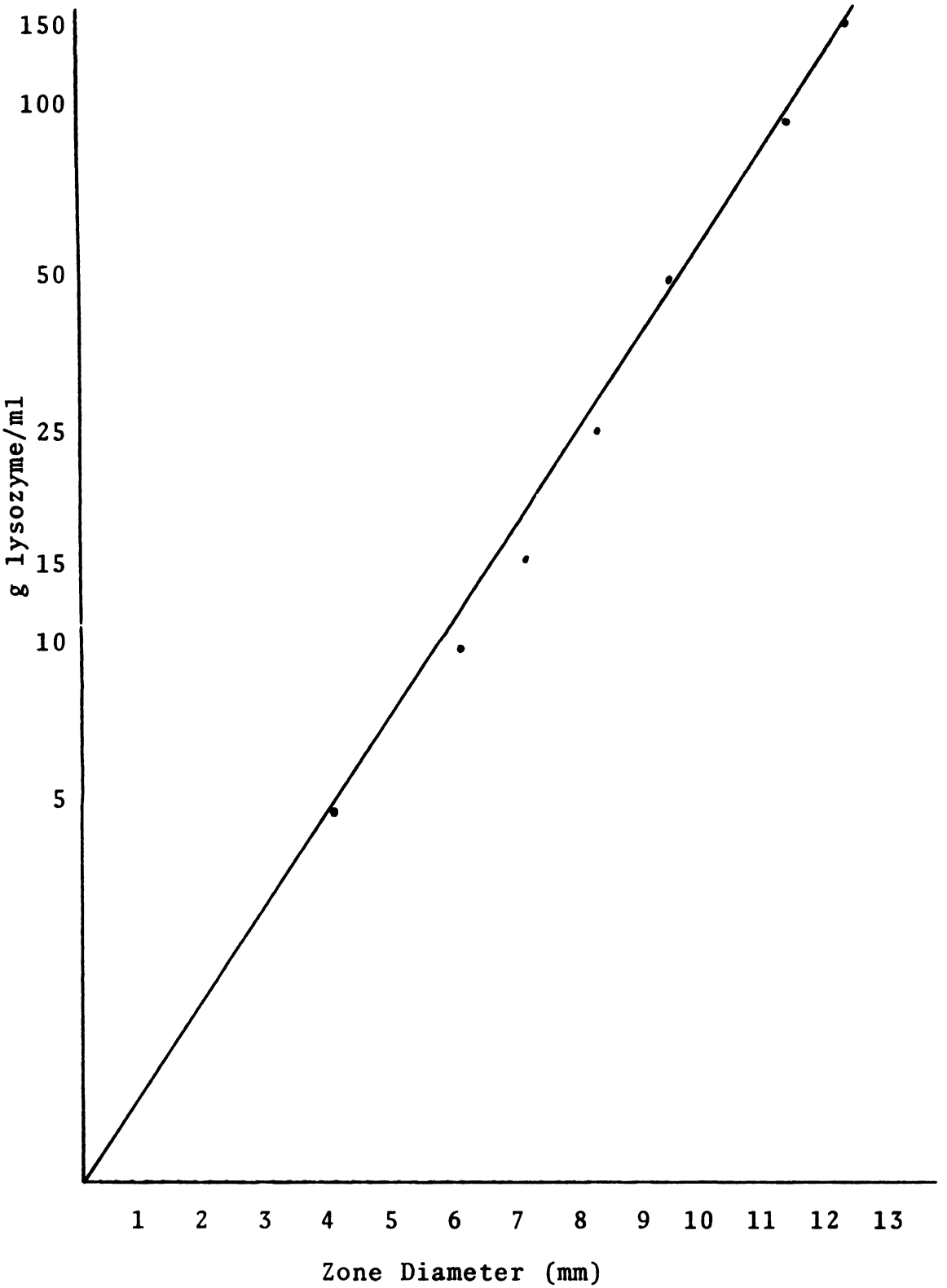

Figure 2. Lysoplate standard curve with human standard.

For comparison purposes, Figure 3 is the lysoplate standard curve with egg-white lysozyme standard. It is very similar to the human lysozyme standard curve, as was also noted by Hansen and Andersen (1973). This is not what one would expect since the human standard is approximately three times more active than the egg-white standard. Zucker et al. (1970) explained this by noting that using the same enzyme standard, lysozyme activity determined with the turbidimetric method was shown to be about one-third of the activity found with the lysoplate method.

Tables 1, 2 and 3 list the results of additional laboratory tests which were done when the test specimens were drawn. The data are included to provide background information to aid in the evaluation of the test results.

Table 1 lists the BUN (blood urea nitrogen) values which were obtained from the SMA-12 reports. Tables 2 and 3 list the creatinine values which I determined using the Jaffe reaction (Davidsohn and Wells, 1962). The purpose of the tests was to ascertain that renal function was within normal limits.

A peripheral white blood count and differential were done on each specimen. Essentially the WBC counts were within normal limits, and the differentials were of normal percentage distribution. In the table the letter "G" refers to granulocytes, which is used as an inclusive term for neutrophils, basophils, and eosinophils. In a few patients with malignant disease there were occasional young neutrophils (myelocytes) present.

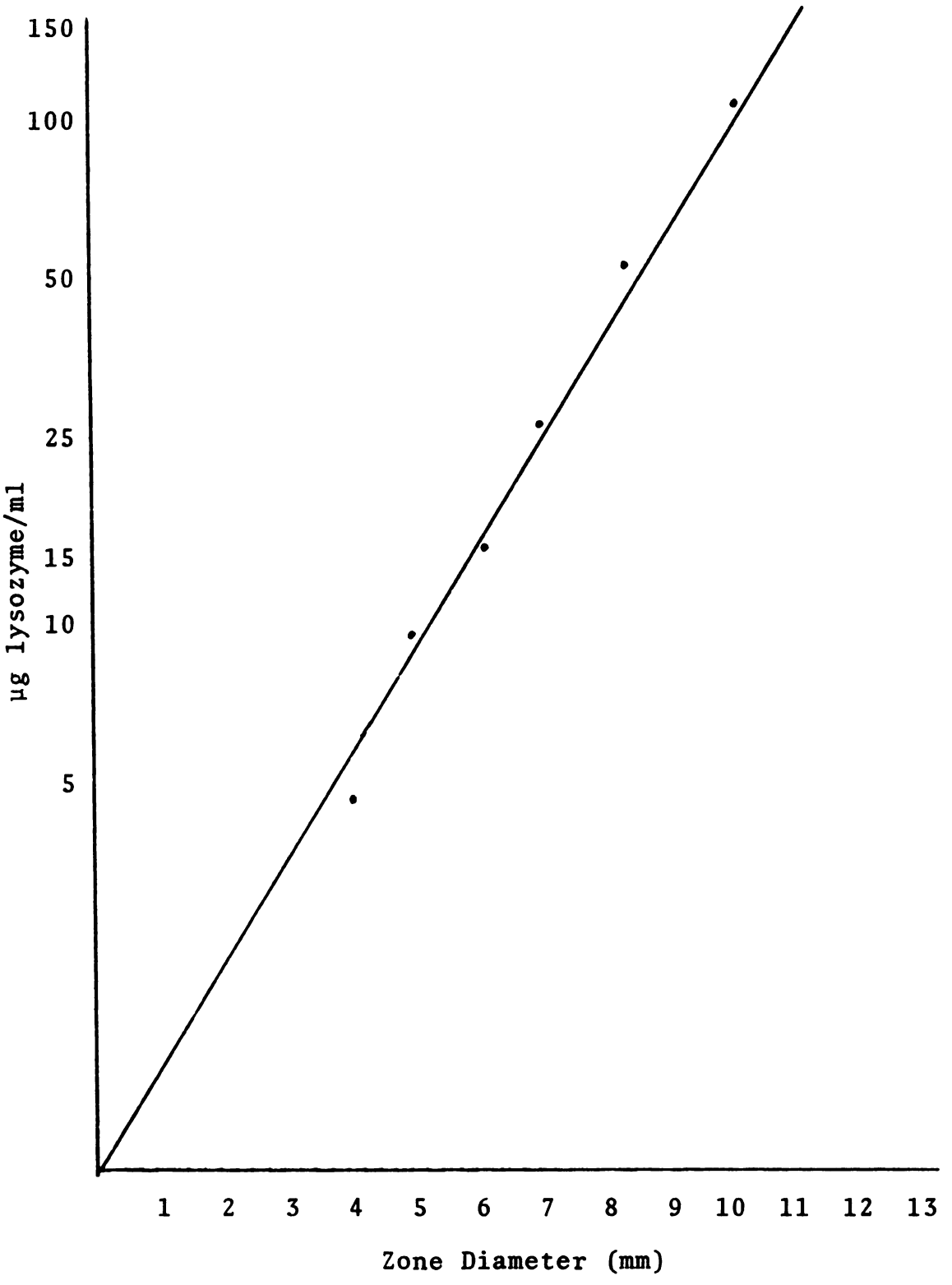

Figure 3. Lysoplate standard curve with egg-white standard.

Table 1. Background laboratory data of patients with malignant disease

	Age/Sex	BUN (mg/dl)	Peripheral WBC/cu.mm.	Peripheral Differential	Clinical Diagnosis
1.	41/M	10	6200	G62 L29 M9	CA of lung
2.	74/M	12	6800	G78 L19 M3	Squamous cell CA
3.	49/M	18	6300	G73 L15 M11	Hepatoma
4.	59/M	15	8500	G67 L26 M7	CA of prostate
5.	70/F	13	5400	G90 L8 M2	CA of breast
6.	62/M	19	7600	G91 L5 M4	CA of esophagus
7.	37/F	14	9800	G62 L34 M4	CA of breast
8.	59/M	10	9300	G75 L16 M9	CA of lung
9.	64/F	9	11500	G74 L20 M6	CA of colon
10.	64/F	17	4400	G64 L32 M4	CA of breast
11.	50/F	19	8890	G76 L19 M5	Broncho-genic CA
12.	64/F	8	9000	G88 L10 M12	Metastatic breast CA
13.	76/M	19	170,000	G5 L95 M0	CLL

Table 2. Background laboratory data for normal individuals

	Age/Sex	Creatinine (mg/dl)	Peripheral WBC/cu.mm.	Peripheral Differential
1.	20/F	0.6	7800	G67 L29 M4
2.	27/M	1.1	6200	G64 L31 M15
3.	55/M	1.2	6500	G66 L29 M5
4.	50/F	0.5	6400	G63 L34 M4
5.	58/F	1.0	5000	G59 L35 M6
6.	40/F	0.9	7400	G53 L38 M9
7.	34/F	1.1	5100	G59 L33 M8
8.	44/F	1.2	8100	G70 L25 M5
9.	21/F	1.1	10600	G85 L12 M3
10.	30/F	1.0	5000	G58 L34 M4
11.	45/F	0.6	7000	G59 L33 M8
12.	27/M	0.8	4000	G50 L40 M10
13.	59/M	1.6	5300	G77 L14 M9
14.	55/F	1.0	7500	G62 L36 M2

Table 3. Background laboratory data on patients with benign disease

	Age/ Sex	Creatinine (mg/dl)	Peripheral WBC /cu.mm.	Peripheral Differential	Disease
1.	65/M	1.0	11000	G70 L20 M10	Coronary insuff.
2.	27/F	1.2	5200	G61 L36 M3	Seborrhea dermatitis
3.	24/F	1.4	6500	G67 L30 M3	Asthma
4.	63/F	0.6	7300	G64 L28 M8	Allergies
5.	54/F	1.3	7400	G63 L29 M8	Arthritis
6.	40/M	0.9	8700	G74 L24 M2	Diabetes
7.	30/M	1.1	6500	G76 L21 M3	Allergies
8.	23/F	1.2	10000	G72 L22 M6	Crohn's disease
9.	40/F	0.8	7300	G64 L32 M4	Hypothyroidism
10.	45/F	2.0	5000	G58 L37 M5	Arteriosclerosis
11.	53/M	0.6	6100	G53 L37 M10	Coagulopathy

Diagnosis, age and sex of each patient are also noted in the table.

The SMA-12 reports were essentially normal with one frequent exception: an elevated LDH (lactic acid dehydrogenase). This is due to the increased cell destruction occurring as a result of the malignant disease process.

Patient #13 in Table 1 is of interest. He was included because of his high concentration of lymphocytes. When the lysozyme level was determined on his cell preparation it was 0 µg lysozyme by both the lysoplate and the turbidimetric methods. This substantiates the published data stating that lymphocytes do not contain lysozyme.

Table 4 lists the volume of the final cell preparation and the Wright's stained differential results. The differentials listed are the average of a minimum of three differentials per cell preparation.

From the data in Table 4 it can be seen that most of the granulocytes have been removed by the Ficoll-Hypaque process, leaving a product composed primarily of lymphocytes and monocytes. The cell preparation volume was adjusted to 10^7 monocytes per ml in all specimens.

Tables 5, 6 and 7 contain the triplicate readings for each lysozyme determination by the lysoplate method. The values are corrected to eliminate any granulocytic lysozyme that may have been present in the specimen.

Table 8 is a summary table for the three groups indicating the mean for each group and the confidence intervals

Table 4. Monocyte preparations

	Cell Preparation Volume (ml) (10^7 monocytes/ml)	Cell Preparation Differential
	Malignant Group	
1.	0.74	G2 L74 M24
2.	0.60	G2 L64 M34
3.	1.30	G1 L68 M31
4.	0.39	G6 L83 M11
5.	0.36	G1 L77 M22
6.	0.73	G9 L52 M39
7.	0.76	G1 L73 M26
8.	1.45	G10 L50 M40
9.	0.96	G3 L80 M17
10.	0.21	G5 L83 M12
11.	0.48	G2 L72 M26
12.	1.03	G10 L61 M29
	Normal Group	
1.	0.25	G4 L88 M8
2.	0.25	G1 L86 M13
3.	0.66	G7 L81 M12
4.	0.47	G4 L83 M13
5.	0.21	G8 L86 M6
6.	0.43	G3 L90 M7
7.	0.19	G2 L92 M6
8.	0.16	G2 L88 M10
9.	0.30	G5 L80 M15
10.	0.25	G5 L88 M7
11.	0.50	G1 L89 M10
12.	0.14	G8 L78 M14
13.	0.35	G10 L76 M14
14.	0.30	G10 L83 M7
	Benign Group	
1.	0.41	G3 L83 M14
2.	0.21	G3 L89 M8
3.	0.45	G3 L86 M11
4.	0.32	G4 L86 M10
5.	0.97	G6 L73 M21
6.	0.51	G10 L74 M16
7.	0.13	G7 L86 M7
8.	0.21	G9 L80 M11
9.	0.39	G6 L85 M9
10.	0.21	G5 L87 M8
11.	0.28	G1 L91 M8

Table 5. Lysoplate readings, malignant group

	Zone Diameter (mm)	Lysozyme Activity (µg/10^7 monocytes)	Corrected Lysozyme Activity	Average Lysozyme Activity
1.	10.0	64	59	
	10.5	85	78	67
	10.0	70	64	
2.	10.5	75	71	
	10.0	65	61	61
	9.5	54	51	
3.	11.0	89	86	
	12.0	133	130	115
	12.0	134	130	
4.	12.0	150	97	
	12.5	135	120	112
	12.5	135	120	
5.	10.0	68	65	
	11.0	96	92	83
	11.0	96	92	
6.	10.0	61	49	
	10.0	64	52	51
	10.0	64	52	
7.	9.5	51	49	
	10.0	57	55	51
	9.5	51	49	
8.	10.0	66	53	
	10.0	66	53	51
	10.0	60	48	
9.	11.0	106	90	
	12.0	156	133	110
	11.5	126	107	
10.	8.5	34	24	
	9.0	37	26	26
	9.0	37	26	
11.	9.5	47	44	
	9.0	45	42	44
	9.5	47	44	
12.	11.0	89	68	
	11.0	89	68	68
	11.0	89	68	

Table 6. Lysoplate readings, normal group

	Zone Diameter (mm)	Lysozyme Activity ($\mu g/10^7$ monocytes)	Corrected Lysozyme Activity	Average Lysozyme Activity
1.	9.0	46	31	
	9.0	46	31	30
	9.0	41	27	
2.	8.0	31	29	
	8.5	37	34	35
	9.0	46	43	
3.	10.5	82	52	
	11.0	87	55	53
	10.5	82	52	
4.	10.0	56	43	
	10.0	56	43	46
	10.0	68	52	
5.	11.5	125	54	
	11.5	125	54	54
	11.5	125	54	
6.	10.5	84	59	
	10.5	84	59	59
	10.5	84	59	
7.	9.0	39	29	
	8.5	34	26	28
	9.0	39	29	
8.	8.5	37	31	
	8.5	37	31	31
	8.5	37	31	
9.	10.5	84	63	
	10.0	56	42	56
	10.5	84	63	
10.	8.5	37	22	
	8.5	31	18	21
	8.5	37	22	
11.	9.0	37	34	
	9.0	37	34	34
	9.0	37	34	

Table 6 (continued)

	Zone Diameter (mm)	Lysozyme Activity ($\mu g/10^7$ monocytes)	Corrected Lysozyme Activity	Average Lysozyme Activity
12.	10.0	68	43	
	10.0	84	53	50
	10.5	84	53	
13.	9.5	56	33	
	9.5	56	33	35
	10.0	68	40	
14.	11.5	125	51	
	11.0	100	41	47
	11.5	125	51	

Table 7. Lysoplate readings, benign group

	Zone Diameter (mm)	Lysozyme Activity ($\mu g/10^7$ monocytes)	Corrected Lysozyme Activity	Average Lysozyme Activity
1.	10.5	84	69	
	10.0	56	46	54
	10.0	56	46	
2.	9.5	47	34	
	9.5	57	41	36
	9.5	47	34	
3.	8.5	37	29	
	8.5	37	29	29
	8.5	37	29	
4.	10.5	85	61	
	10.5	79	56	59
	10.5	85	61	
5.	10.0	56	44	
	10.0	56	44	44
	10.0	56	44	
6.	10.0	56	34	
	10.0	56	34	34
	10.0	56	34	
7.	8.5	37	10	
	8.5	37	19	20
	9.0	46	23	
8.	10.0	64	38	
	10.0	64	38	38
	10.0	64	38	
9.	10.5	84	50	
	10.5	84	50	47
	10.0	68	41	
10.	9.5	56	34	
	9.5	56	34	32
	9.5	46	28	
11.	8.5	37	33	
	9.5	46	41	36
	8.5	37	33	

Note: It was necessary to use two lot numbers of lysoplates. A standard curve was constructed for each lot number. The curves varied slightly. Therefore a 10 mm zone will give a slightly different value depending upon which lot number was used.

Table 8. Summary of lysoplate determinations

Malignant Group	Normal Individuals	Benign Group
67	30	54
61	35	36
115	53	29
112	46	59
83	54	44
51	59	34
51	28	20
51	31	38
110	56	47
26	21	32
44	34	36
68	50	
	35	
	47	
$\bar{x} = 70$	$\bar{y} = 41$	$\bar{z} = 39$

$S = 19.2$

95% simultaneous confidence intervals:

$$9.59 \leq \mu_1 - \mu_2 \leq 48.41$$

$$10.45 \leq \mu_1 - \mu_3 \leq 51.55$$

$$-17.76 \leq \mu_2 - \mu_3 \leq 21.76$$

μ_1 = population mean of malignant group

μ_2 = population mean of normal individuals

μ_3 = population mean of benign group

calculated according to Scheffé simultaneous 95% confidence intervals.

Since the population mean of the malignant group minus the population mean of the normal group in the 95% simultaneous confidence intervals does not contain zero, it indicates that the population mean for the malignant group is larger than the population mean for the normal group. A similar method of interpretation is used for comparing the population mean of the malignant group minus the population mean of the benign group in the 95% simultaneous confidence intervals.

Since the population mean of the normal group minus the population mean of the benign group in the 95% simultaneous confidence interval does contain zero, it indicates that the population means of the two groups could be the same.

The cell preparation data were also tested by the null hypothesis, which also concluded that the population mean of the malignant group was larger than the population mean of the normal group. The descriptive level at which we would reject the null hypothesis was less than .0005.

Figure 4 is a photograph of a lysoplate showing the clear area of lysis due to the activity of lysozyme on the *Micrococcus lysodeikticus* substrate.

Figure 5 is a photograph of a Wright's stained smear of the monocyte preparation.

Figure 4. Lysoplate photograph.

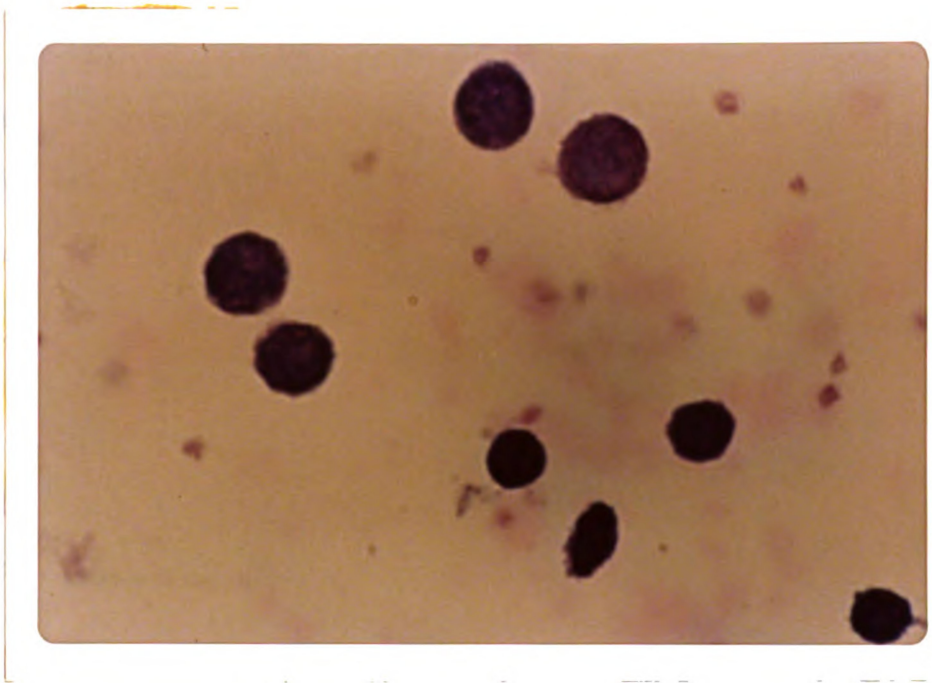

Figure 5. Monocyte cell preparation. Wright's-Giemsa stain X 1000.

Serum Lysozyme as Measured by the Turbidimetric Method

The turbidimetric method is the one of choice for measuring specimens with lysozyme concentrations below 15 µg/ml as is usually the case with human blood serum.

The egg-white lysozyme standard is used with this method because the human lysozyme standard is too active. The 5 µg/ml human standard has a ΔA of .074, and the 10 µg/ml has a ΔA of .112, which indicates the substrate has been depleted. Figure 6 is the standard curve for the turbidimetric method using an egg-white standard.

Table 9 lists the data of the triplicate determinations of each specimen and the average µg/ml of lysozyme for the three readings is noted.

Please note in regard to interpretation of the ΔA quantities that the values were read from the curve for its own substrate lot number. Two lots of substrate were used and the curves were slightly different.

The serum lysozyme values for the three groups are very similar and do not seem to be affected by the malignant process. The means for the respective groups and the 95% simultaneous confidence intervals calculated according to Scheffé are as follows:

	Mean	95% simultaneous confidence intervals
Malignant	12.5	$-2.96 \leq \mu_1 - \mu_2 \leq 5.56$
Normal	11.5	$-2.80 \leq \mu_1 - \mu_3 \leq 6.20$
Benign	11.1	$-3.93 \leq \mu_2 - \mu_3 \leq 4.73$

S 4.21 μ_1 = population mean of malignant group

μ_2 = population mean of normal group

μ_3 = population mean of benign group

Since each of the three confidence intervals contains a zero, it is possible that the population mean could be the same for each of the three groups.

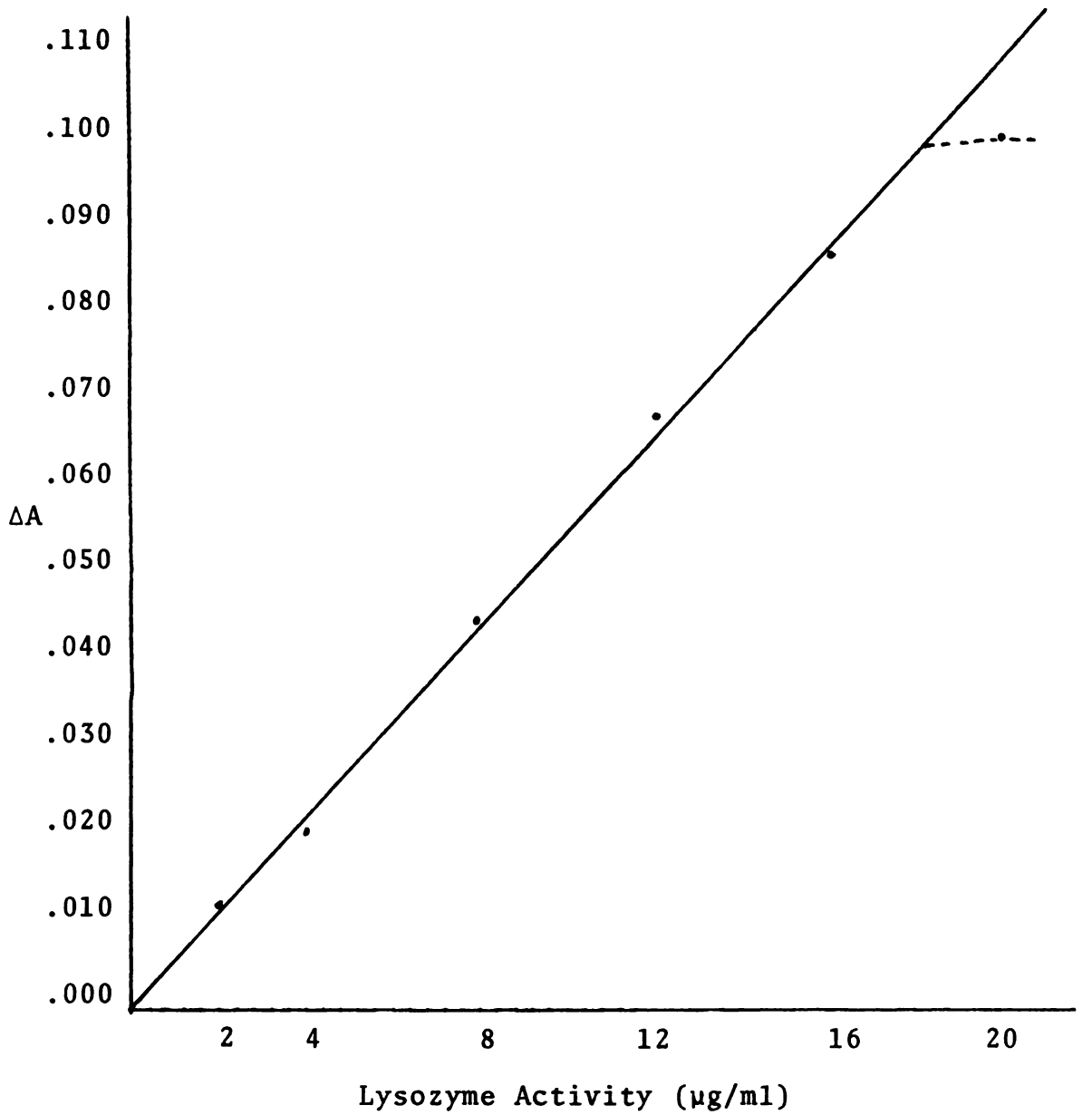

Figure 6. Turbidimetric standard curve with egg-white standard.

Table 9. Serum lysozyme readings by the turbidimetric method

	Malignant		Normal		Benign	
	ΔA	µg/ml	ΔA	µg/ml	ΔA	µg/ml
1.	.064		.046		.073	
	.066	12.0	.049	8.5	.074	13.1
	.065		.047		.073	
2.	.052		.074		.043	
	.054	9.8	.074	10.2	.047	8.2
	.053		.074		.045	
3.	.076	14.7 x 2	.060		.046	
	.084	29.4	.062	8.6	.047	8.3
	.080		.061		.046	
4.	.079		.078		.059	
	.085	14.9	.079	14.0	.061	10.8
	.081		.078		.060	
5.	.050		.100		.100	
	.054	9.6	.098	13.8	.098	13.2
	.052		.099		.099	
6.	.042		.093		.060	
	.042	7.8	.093	12.9	.058	8.0
	.042		.093		.059	
7.	.051		.062		.087	
	.050	9.3	.063	11.1	.088	11.5
	.050		.062		.086	
8.	.059		.092		.095	
	.060	11.1	.090	12.6	.096	12.6
	.060		.091		.094	
9.	.054		.110		.080	
	.054	10.0	.113	20.0	.080	10.6
	.054		.111		.081	
10.	.037		.064		.083	
	.035	6.7	.065	9.0	.084	11.1
	.036		.065		.084	
11.	.078		.046		.101	
	.081	14.7	.049	8.5	.103	14.4
	.080		.047		.101	

Table 9 (continued)

	Malignant		Normal		Benign	
	ΔA	µg/ml	ΔA	µg/ml	ΔA	µg/ml
12.	.096		.054			
	.100	18.0	.053	9.7		
	.098		.054			
13.	.048		.079			
	.048	8.9	.078	10.5		
	.048		.079			
14.			.083			
			.083	11.5		
			.083			

SUMMARY AND CONCLUSIONS

The intracellular monocytic lysozyme level is elevated in patients with untreated malignant disease. Measurements were made using the lysoplate technique and a human lysozyme standard.

The serum lysozyme level does not appear to be affected by the malignant disease process. Measurements were made using the turbidimetric method and egg-white lysozyme standard.

We now know that monocytes and macrophages are selectively cytotoxic for some tumor cells, and the concentration of intracellular monocytic lysozyme in individuals with malignant disease is higher than in normal control individuals. The most significant question now is: Is the monocytic-macrophage intracellular lysozyme the actual agent that is destroying tumor cells?

Additional studies need to be conducted to determine the mechanism by which lysozyme may act on the tumor cell substrate.

APPENDICES

APPENDIX A

REAGENTS

Ficoll-Hypaque Solution

Ficoll	9.54 gm
Hot dist. water	106.00 ml
Hypaque (33.9%)*	44.00 ml

Add hot distilled water to Ficoll. Bring to a boil.

Remove from heat and allow to cool slightly.

Add Hypaque (33.9%) and mix.

Refrigerate. Stable for two weeks.

Sodium Phosphate Buffer for Lysoplate Standard Diluent

$NaH_2PO_4 \cdot H_2O$	6.9 gm

Dissolve sodium phosphate in one liter of distilled water.

pH to 7.0. Refrigerate.

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*50% Hypaque 30.0 ml
dist. water 14.0 ml

APPENDIX B

INFORMED WRITTEN CONSENT FORM

1. A registered medical techologist will draw 55 ml of blood (approximately 2 fluid ounces) into a 60 ml plastic syringe by a single venipuncture technique. One kind of white blood cell, the monocyte, will be analyzed for its lysozyme content.

2. The attendant discomfort and risk are the same as for a routine venipuncture.

3. There is no direct benefit to the blood donor. However, the knowledge obtained from this research may aid in the diagnosis and treatment of disease.

4. I understand that I am free to withdraw my consent and to discontinue my participation in the project.

5. I understand the identity of the specimens will remain anonymous, and that the confidentiality of the reports will be maintained at all times. Should the results be published, the identity of the specimens will not be revealed.

I freely consent to donate my blood specimen for this research. I understand all of the above statements and all of my questions concerning the procedure have been adequately answered.

Signed_____

Date_____

Witness_____

Physician signature_____

REFERENCES

REFERENCES

Ambrose, E. J.: *The Biology of Cancer.* Van Nostrand-Reinhold, Princeton, NJ, 1966.

Arnon, R., and Sela, M.: Antibodies to a unique region in lysozyme provoked by a synthetic antigen conjugate. Proceedings of National Academy of Science, 62, (1969): 163-170.

Bluming, A. Z., Vogel, C. L., Ziegler, J. L., Mody, N., and Kamya, G.: Immunological effects of BCG in malignant melanoma: Two modes of administration compared. Annals of Internal Medicine, 76, (1972): 405-411.

Boyum, A.: Separation of leucocytes from blood and bone marrow. Scandinavian Journal of Clinical and Laboratory Investigation, 21, (1968): suppl. 97.

Brodersen, M. P., and Burns, C. P.: The separation of human monocytes from blood including biochemical observations. Proceedings of the Society for Experimental Biology and Medicine, 144, (1973): 941-944.

Canfield, R. E., and Liv, A. K.: The disulfide bonds of egg white lysozyme (muramidase). Journal of Biological Chemistry, 240, (1965): 1997-2002.

Carson, M.E., and Dannenberg, A. M.: Hydrolytic enzymes of rabbit mononuclear exudate cells. II. Lysozyme: Properties and quantitative assay in tuberculosis and control inbred rabbits. Journal of Immunology, 94, (1965): 99-104.

Cohn, Z. A., and Wiener, E.: The particulate hydrolases of macrophages. Journal of Experimental Medicine, 118, (1963): 991-1020.

Currie, G. A., and Bagshave, K. D.: Active immunotherapy with *C. parvum* and chemotherapy in murine fibrosarcomas. British Medical Journal, 1, (1970): 541-544.

Daniels, J. C.: Lysozyme. In *Clinical Chemistry Principles and Technics.* Ed. by Henry, R. J., Cannon, D. C., and Winkelman, J. W., (1964), Harper and Row, NY.

Dimitrov, N., and Nodine, J.: *Drugs in Hematologic Reactions. 29th Hahnemann Symposium.* Grune and Stratton, Inc., NY, 1974.

Eisen, H. N.: *Immunology.* Harper and Row, NY, 1974.

Farhangi, M., and Osserman, E. F.: *De novo* synthesis of lysozyme by bone marrow cells of a patient with monomyelocytic leukemia. In *Lysozyme.* Ed. by Osserman, E. F., Canfield, R. E., and Beychok, S., (1974), Academic Press, NY.

Finch, S. C., Castro, O., Lippman, M. E., Donadio, J. A., and Perillie, P. E.: Lysozyme in leukopathic states. In *Lysozyme.* Ed. by Osserman, E. F., Canfield, R. E., and Beychok, S., (1974), Academic Press, NY.

Flanagan, P., and Lionetti, F.: Lysozyme distribution in blood. Blood, 10, (1955): 497-501.

Fleming, A.: On a remarkable bacteriolytic element found in tissues and secretions. Proceedings of the Royal Society of London, B93, (1922): 306-317.

Gordon, S., Todd, J., and Cohn, Z.: *In vitro* synthesis and secretion of lysozyme by mononuclear phagocytes. Journal of Experimental Medicine, 139, (1974): 1228-1248.

Halpern, B. N.: Role du systeme reticulo-endothelial dans l'immunite anti-bacterienne et anti-tumorale. CNRS, 115, (1953), Paris.

Hamlin, I. M. E.: Possible host resistance in carcinoma of the breast: A histological study. British Journal of Cancer, 22, (1968): 383-401.

Hansen, N.E., and Andersen, V.: Lysozyme activity in human neutrophilic granulocytes. British Journal of Haematology, 24, (1973): 613-623.

Holterman, O.A., Casale, G. P., and Klein, E.: Tumor cell destruction by macrophages. Journal of Medicine Experimental and Clinical, 3, (1972): 305-309.

Holterman, O. A., Klein, E., and Casale, G. P.: Selective cytotoxicity of peritoneal leucocytes for neoplastic cells. Cellular Immunology, 9, (1973): 339-352.

Holterman, O.A., Djerassi, I., Lisafeld, B. A., Elias, E. G., Papermaster, B. W., and Klein, E.: *In vitro* destruction of tumor cells by human monocytes. Proceedings of the Society for Experimental Biology and Medicine, 147, (1974): 456-459.

Jolles, J., Sportorno, G., and Jolles, P.: Lysozymes characterized in duck egg-white. Nature (London), 208, (1965): 1204-1205.

Jolles, P., Jauregui-Adell, J., and Jolles, J.: Le lysozyme de blanc d'oeuf de poule. Comptes Rendus Hebdomadaires Des Seances De L'Academie Des Sciences, 258(2), (1964): 3926-3928.

Jolles, P.: Lysozymes: A chapter of molecular biology. Angewandte Chemie International Edition, 8, (1969): 227-239.

Klein, E.: Hypersensitivity reactions at tumor sites. Cancer Research, 29, (1969): 2351-2362.

Lessin, L. S., and Bessis, M.: Morphology of monocytes and macrophages. In *Hematology*. Ed. by Williams, W. J., Beutler, E., Erslev, A. J., and Rundles, R. W., (1972), McGraw-Hill, NY.

Moreland, F. B.: Clinical chemistry. In *Clinical Diagnosis by Laboratory Methods*. Ed. by Davidsohn, I. and Wells, B., (1962), W. B. Saunders Co., Philadelphia.

Mouton, A., and Jolles, J.: On the identity of human lysozymes isolated from normal and abnormal tissues or secretions. Federation of European Biochemical Societies, 4, (1969): 337-340.

Myrvik, Q. N., Leake, E. S., and Fariss, B.: Lysozyme content of alveolar and peritoneal macrophages from the rabbit. Journal of Immunology, 86, (1961): 133-136.

Myrvik, Q. N., Leake, E. S., and Oshima, S.: A study of macrophages and epithelioid-like cells from granulomatous (BCG-induced) lungs of rabbits. Journal of Immunology, 89, (1962): 745-751.

Ohta, H., and Osserman, E. F.: Hydrolytic enzymes of human blood monocytes and neutrophils with special reference to lysozyme (muramidase). Tohoku Journal of Experimental Medicine, 107, (1972): 229-240.

Old, L. J., Benacerraf, B., Clarke, D. A., Carswell, E. A., and Stockart, E.: The role of the reticuloendothelial system in the host reaction to neoplasia. Cancer Research, 21, (1961): 1281-1300.

Oshima, S., Myrvik, Q. N., and Leake, E.: The demonstration of lysozyme as a dominant tuberculostatic factor in extracts of granulomatous lungs. British Journal of Experimental Pathology, 42, (1961): 138-144.

Osserman, E. F., and Lawlor, D. P.: Serum and urinary lysozyme (muramidase) in monocytic leukemia. Journal of Experimental Medicine, 124, (1966): 921-955.

Osserman, E. F.: Note on nomenclature. In *Lysozyme*. Ed. by Osserman, E. F., Canfield, R. E., and Beychok, S., (1974), Academic Press, NY.

Osserman, E. F., Klockars, M., Halper, J., and Fischel, R. E.: Studies of the effects of lysozyme on mammalian cells. In *Lysozyme*. Ed. by Osserman, E. F., Canfield, R. E., and Beychok, S., (1974), Academic Press, NY.

Papermaster, B. W., Holterman, O.A., Rosner, D., Klein, E., and Dao, T.: Regressions produced in breast cancer lesions by a lymphokine fraction from a human lymphoid cell line. Research Communications in Chemical Pathology and Pharmacology, 8, (1974): 413-416.

Perillie, P. E., Kaplan, S., Lefkowitz, E., Rogaway, W., and Finch, S.: Studies of muramidase (lysozyme) in leukemia. Journal of the American Medical Association, 203, (1968): 317-322.

Perillie, P. E., and Finch, S.: Lysozyme measurements in acute leukemia: Diagnostically useful? In *Lysozyme*. Ed. by Osserman, E. F., Canfield, R. E., and Beychok, S., (1974), Academic Press, NY.

Phillips, D. C.: The hen egg-white lysozyme molecule. Proceedings of the National Academy of Sciences, 57, (1967): 484-495.

Prager, E. M., and Wilson, A. C.: The dependence of immunological cross reactivity upon sequence resemblance among lysozymes. Journal of Biological Chemistry, 246, (1971): 5978-5989.

Rabinowitz, Y.: Adherence and separation of leukemic cells on glass bead columns. Blood, 26, (1965): 100-103.

Senn, H. J., Chu, B., O'Malley, J., and Holland, J. F.: Experimental and clinical studies on muramidase (lysozyme). Acta Haematologica, 44, (1970): 65-77.

Yashphe, D.: Immunological factors in nonspecific stimulation of host resistance to syngeneic tumors. In *Immunological Parameters of Host-Tumor Relationships*. Ed. by Weiss, D. W., (1974), Academic Press, NY.

Zbar, B., and Tanaka, T.: Immunotherapy of cancer: Regression of tumors after intralesional injection of living *Mycobacterium bovis*. Science, 172, (1971): 271-273.

Zbar, B.: Immunotherapy of guinea pig cancer with BCG. In *The Role of Immunological Factors in Viral and Oncogenic Processes*. Ed. by Beers, R. F., Tilghman, R. C., and Basset, E. G., (1974), Johns Hopkins University Press, Baltimore.

Zucker, S., Hanes, D. J., Vogler, W. R., and Eanes, R.: Plasma muramidase: A study of methods and clinical applications. Journal of Laboratory and Clinical Medicine, 75, (1970): 83-92.

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